

**REMARKS**

After entry of the amendments made herein, claims 1 – 13 are pending in the application. Claims 1 and 12 have been amended. Claims 2, 3, 9, and 13 - 32 have been cancelled. New claims 33 and 34 have been added. No new matter has been added by virtue of the amendments, support being found throughout the specification and from the claims as filed.

Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

**Claim Rejections 35 USC 112**

The Examiner has indicated that claims 1 – 13 have been rejected under 35 USC 112, first paragraph, because the specification, while being enabling for 1) a method of reducing myotonia in the muscle of an individual suffering from myotonia, comprising *intramuscular injection* of a recombinant adeno-associated viral (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding muscleblind (MBNL1) protein, wherein expression of the MBNL1 protein results in reducing myotonia in the muscle of the individual, and 2) a pharmaceutical composition comprising a recombinant adeno-associated viral (rAAV) vector comprising a *promoter operably linked* to a nucleic acid encoding mucleblind (MBNL1) protein, but the specification does not reasonably provide enablement for 1) any mode of administration of the rAAV; 2) utilizing any combination of transgenes encoding for MBNL1, MBNL2, MBNL3; 3) the reversing of mis-splicing of variously claimed proteins in vivo. Applicants respectfully disagree.

The present claims recite a method of treating myotonic dystrophy in a subject, comprising administering by intramuscular injection to a mammal in need thereof, a therapeutically effective amount of recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding a MBNL1 protein, wherein expression of the protein results in reducing myotonic dystrophy in the subject.

Without acquiescing to any validity of the Examiner's arguments, and solely in the interest of advancing prosecution, Applicants have amended the claims to recite that the mode of administration is intramuscular and that the rAAV vector comprises a promoter operably linked to a nucleic acid encoding a MBNL1 protein.

The specification provides guidance and support for treating myotonic dystrophy by administering by intramuscular injection to a mammal in need thereof, a therapeutically effective amount of recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding a MBNL1 protein, wherein expression of the protein results in reducing myotonic dystrophy in the subject.

As taught in the specification at page 3, beginning at line 6, myotonia is a characteristic of myotonic dystrophy, where heart block, ocular cataracts, hypogonadism, and nervous system dysfunction are all manifestations of the disease :

Myotonic dystrophy (dystrophia myotonica, DM) is a multisystemic, dominantly inherited disorder often characterized by **myotonia**, or, delayed muscle relaxation due to repetitive action potentials in myofibers, and muscle degeneration. Manifestations of DM may also include **heart block, ocular cataracts, hypogonadism, and nervous system dysfunction**.

Applicants teach in the Examples that MBNL1 contributes to DM pathogenesis in mice with a targeted deletion of Mbnl1 exon3. In Example 2 on page 27, Applicants teach that the Mbnl1 exon3 mice show myotonia, a characteristic of DM, and develop "**distinctive ocular cataracts** that progress from subcapsular 'dust-like' opacities to **mature cataracts (that) are a prominent DM-associated feature.**" (p. 28, lines 27 – 28; emphasis added). Further, Applicants investigate alternative splicing related to MBNL, and show that pre-mRNA targets that are known to be misregulated in DM striated muscle are regulated by MBNL. For example, on page 32 beginning at line 32, Applicants teach that the MBNL family regulates human IR:

Another pre-mRNA target that is misregulated in DM striated muscle is the IR (Savkur R S, Philips A V, Cooper T A (2001), Nat Genet 29: 40-47; Savkur R S, Philips A V, Cooper T A, Dalton J C, Moseley M L, Ranum L P, Day J W (2004), Am J Hum Genet 74: 1309-1313). To test whether the MBNL family can also regulate human IR, the three MBNL family members were co-expressed with a human IR minigene. In contrast to the inhibitory effect of MBNL on cTNT splicing, coexpression of MBNL family members with an IR minigene strongly induces exon inclusion, whereas GFP alone had no effect (FIG. 6C).

In Example 9, Applicants describe fluorescence in situ Hybridization (FISH) and immunofluorescence (IF) Analysis of DM1 Brain. Fluorescence in situ hybridization (FISH) of brain sections with CAG repeat probes revealed nuclear RNA foci in every individual with DM1 (n=10, FIG. 12A) but not in controls with (n=7) or without (n=6) neurologic disease. (p.39, line 34). Following FISH, sections were incubated with primary antibodies, including MBNL1. MBNL1 fluorescence intensity (mean optical density in monochrome mode in arbitrary units) in the region of interest was determined for 20 cortical neuronal nuclei per subject. Applicants report on p. 42, lines 18 – 23:

Counts of 100 NeuN-positive cells from temporal and frontal cortex of 4 patients with classical DM1 (selected for best relative preservation of cortical architecture) showed RNA foci in >85% of cortical neurons in each case. More than one focus was visible in about 30% of cortical neurons, and occasional neurons had up to 15 small foci. In contrast, the individual having a small CTG repeat expansion (77 repeats) and mild phenotype (cataracts, mild weakness, and cognitive impairment after age 60 years) had foci in only 39% of NeuN-positive neurons in temporal cortex.

Clearly, the specification provides guidance and support that treatment with MBNL1, as claimed, results in reduction of DM.

The Examiner argues that the specification does not provide enablement for “the reversing of mis-splicing of variously claimed proteins *in vivo*.” (Office Action, p.3). The Examiner argues that “(t)he working examples that pertain to examples 4 – 8 are *in vitro* examples, with cell types that are not necessarily representative of cells that are isolated from a mammal suffering from an aberrant microsatellite expansion disease.” (Office Action, p.7). The Examiner argues that “the specification uses primary chicken skeletal muscle cells (Example 5), which are made to express human and chicken cTNT and human IR minigenes; however these cells are not isolated from a mammal that has an aberrant microsatellite disease, therefore it is unclear what nexus can be concluded from these *in vitro* results and a method of treating an aberrant microsatellite expansion disease *in vivo*.” (Office Action, p.7). Applicants disagree.

The specification teaches at p.3, line 32, that “misregulated alternative splicing in DM1 has been demonstrated for six pre-mRNAs: **cardiac troponin T (cTNT), insulin receptor (IR)**, muscle-specific chloride channel (CIC-1), tau, myotubularin-related protein 1 (MTMR1) and fast skeletal troponin T (TNNT3) (Kanadia R N, Johnstone K A, Mankodi A, Lungu C, Thornton C A, Esson D, Timmers A M, Hauswirth W W, Swanson M S (2003), Science 302: 1978-1980).” (emphasis added). The also teaches that specification teaches that “the insulin resistance and myotonia observed in DM1 correlate with the disruption of splicing of two pre-mRNA targets, **IR** and CIC-1, respectively (Savkur R S, Philips A V, Cooper T A, Dalton J C, Moseley M L, Ranum L P, Day J W (2004), Am J Hum Genet 74:1309-1313).” (p.4, line 3 – 7; emphasis added). Applicants teach at p. 4, beginning at line 23, that “(f)urthermore, cTNT minigenes expressed in DM1 muscle cultures or cTNT and IR pre-mRNAs co-expressed with CUG repeat RNA in normal cells reproduce the aberrant splicing patterns observed for endogenous genes in DM cells (Philips A V, Timchenko L T, Cooper T A (1998), Science 280: 737-741; Savkur R S, Philips A V, Cooper T A (2001), Nat Genet 29: 40-47).”

Further, referring to Example 5, Applicants point out that, given the teaching in the specification, the experiments described in Example 5 were carried out to determine whether MBNL proteins can alter the splicing patterns of pre-mRNAs known to be abnormally regulated in DM1 striated muscle.” (p.31, line 15). Accordingly, human and

chicken cTNT and human IR minigenes were expressed with or without each of the three GFP-MBNL fusion proteins or with GFP alone. Applicants show that GFP-MBNL1, 2 and 3 strongly repressed inclusion of both human and chicken cTNT exon 5 in primary chicken skeletal muscle cultures, while expression of GFP to levels comparable to, or greater than, GFP-MBNL fusion proteins had no effect on splicing (FIGS. 6A and 6B and p. 32) and that in contrast to the inhibitory effect of MBNL on cTNT splicing, coexpression of MBNL family members with an IR minigene strongly induces exon inclusion, whereas GFP alone had no effect (FIG. 6C and p. 33).

Applicants again point out Example 4 beginning at page 30 that shows AAV-MBNL1 Injection and Clcn1 splicing. In these experiments, the left tibialis anterior muscle of mice were injected with a recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding MBNL1 protein (rAAVMyc-hMBNL1) and the left and right TAs were collected for total RNA preparation and assayed for recovery of the normal Clcn1 pre-mRNA splicing pattern. The results are shown in Figure 5A. The results show that the levels of the abnormal splicing products were decreased, **while the level of the normal splicing product was increased**, following rAAV1Myc-hMBNL1 injection.

Accordingly, Applicants have taught that misregulated alternative splicing of certain mRNAs has been reported in DM1, and further, Applicants have shown that MBNL proteins regulate specific mRNA targets, namely cTNT and IR minigene. Applicants have established a nexus between the alternative splicing of cTNT and IR minigene by MBNL and a method of treating an aberrant microsatellite expansion disease.

Taken together, the teachings of the specification and knowledge of one of skill in the art enables one of skill in the art to practice the full scope of the claimed invention without having to resort to undue experimentation. Applicants accordingly request that the rejection be reconsidered and withdrawn.

### **Claim Rejections 35 USC 103(a)**

The Examiner has indicated that claims 12 – 13 have been rejected under 35 USC 103(a) as being unpatentable over Snyder et al. (Human Gene Therapy (1997)) when taken with Miller et al. (EMBO J., 2000) as evidenced by the Uniprot website “MBNL1” accessed online May 10, 2009. (Office Action, p.10). Applicants respectfully disagree.

The Examiner argues that “Snyder teach utilizing a recombinant rAAV vector for gene transfer into adult immunocompetent mice and teach that AAV vectors efficiently and stably transduce post-mitotic muscle fibers and myoblasts in vivo.” (Office Action, p.11). The Examiner admits that “Snyder do not specifically teach that the rAAV vector contains a transgene that encodes for MBNL1 (but) prior to the invention Miller teach the sequence of MBNL1 and...that MBNL1 is expressed in muscle tissue.” (Office Action, p.11). Thus, the Examiner argues that “it would have been obvious...to modify the teachings of Snyder to produce an rAAV vector containing a transgene that encoded MBNL1 with a reasonable expectation of success (and) one of ordinary skill in the art would have been motivated to make this modification to test the ability of the rAAV vector to express MBNL, to study MBNL overexpression in vivo.” (Office Action, p.12).

The present claims are directed to a pharmaceutical composition comprising a recombinant adeno-associated virus (rAAV) vector comprising a promoter operably linked to a nucleic acid encoding MBNL1 protein. (claim 12).

Synder does not teach or suggest a rAAV vector comprising a promoter operably linked to a nucleic acid encoding MBNL1 protein that can be used therapeutically. The Miller reference does not cure the defects of the Snyder reference. The Miller reference is directed to the identification of triplet repeat expansion (EXP) double-stranded (ds) RNA-binding proteins, which selectively associate with DM1 expansions. The Miller reference teaches purifying the EXP proteins and matches were found to the human 42 kDa MBNL protein. (p.4441). At best, the Miller reference teaches that “ EXP proteins were structurally related to the Drosophila mbl proteins and expressed at a high level in skeletal muscle (which) suggested that EXP proteins might play key roles during the terminal differentiation of mammalian cell precursors.” (p.4442). Nowhere does the

Miller reference teach MBNL1 would be useful **in a pharmaceutical composition for therapeutic administration.**

Accordingly, it would not have been obvious to modify the teachings of Snyder to produce an rAAVV vector containing a transgene that encoded MBNL1 with a reasonable expectation of success.

Applicants respectfully request that the rejection be withdrawn.

**CONCLUSION**

For the reasons provided, Applicant submits that all claims are allowable as written and respectfully requests early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

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Respectfully submitted,

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